Highly potent depeptidyl peptidase IV inhibitors derived from Alogliptin with the pharmacophore hybridization and lead optimization

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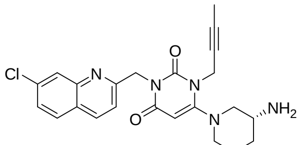
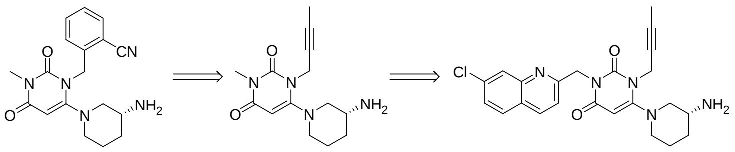
GRAFIC ABSTRACT



11o IC_{50} =0.36 nM
F=73.3%,$T_{1/2}$=5 h



Alogliptin IC_{50} = 1.5 nM

11a IC_{50} =198 nM

11m IC_{50} =0.36 nM
F=73.3%,$T_{1/2}$=5 h

ABSTRACT

The structural superposition of DPP-IV complex with Alogliptin and Linagliptin displayed a similar binding mode. The butynyl of Linagliptin and cyanobenzyl of Alogliptin occupy the S1 pocket which therefore could be mutually switched. Thus a pharmacophore hybridization of Alogliptin was initiated and led to a novel DPP-IV inhibitor **61**. Though it did not exhibit desired activity (IC_{50}= 0.2 μM), the butynyl compound acts as a lead compound triggered a following structural optimization. A novel series of potent DPP-IV inhibitors represented by compound **77** (IC_{50}= 0.36 nM) were obtained with a robust pharmacokinetic profile and better *in vitro* and *in vivo* efficacy than Alogliptin.

KEYWORDS

DPP-IV inhibitor, type 2 diabetes, superposition, pharmacophore hybridization, *in vivo*

1. Introduction

Type 2 diabetes (T2D, formerly referred to as non-insulin-dependent or adult-onset diabetes) results from the body's ineffective use of insulin and comprises over 90% of diabetes patients. With more than 371 million people affected worldwide in 2012, diabetes is a big social burden with more than 471 billion dollars cost on its healthcare. Insufficient blood glucose control leads to multi-system complications with severe harm to T2D patients' lives. However traditional treatments, such as secretagogues and insulin sensitizer, are usually associated with undesired side effects like hypoglycemia, edema, body weight gain. Thus, more and persistent effort should be paid to meet the medical need of T2D and relief its global burden[1].

Glucose like peptidase-1 (GLP-1) is an important incretin which contributes to the increase of insulin secretion and sensitivity, β cell mass, and satiety, as well as the reduction of glucagon secretion and gastric

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emptying. Yet GLP-1 is rapidly truncated by dipeptidyl peptidase IV (DPP-IV) and lost its function in the normal physical condition[2]. Thus inhibition of DPP-IV could effectively maintain the GLP-1 function and control glucose level. DPP-IV inhibitors gradually became the major intervention for type 2 diabetics represented by marketed drugs Sitagliptin **1**[3], Vildaglipin **2**[4], Saxagliptin **3**[5], Alogliptin **4**[6], Linagliptin **5**[7], Gemigliptin **6**[8], and Teneligliptin **7**[9] (Figure 1). Compared to conventional treatments, DPP-IV inhibitors have demonstrated themselves as of good patient compliance, reduced risks of hypoglycemia, and less side effect[10].

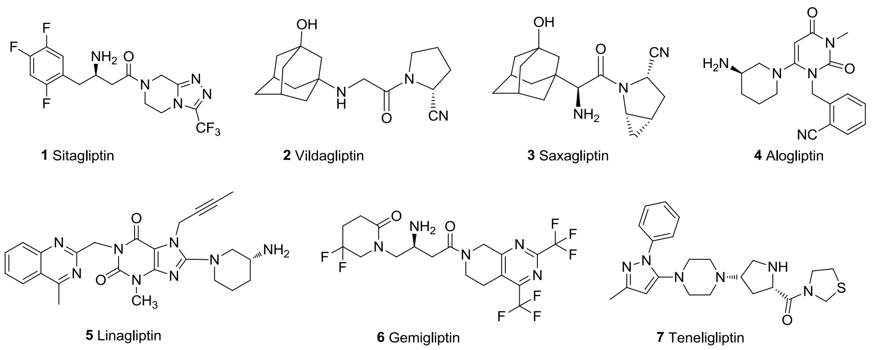
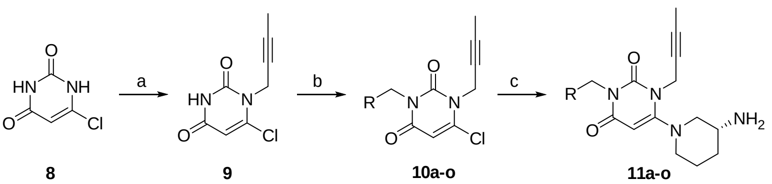


Figure 1. Marketed DPP-IV inhibitors.

In our previous work, we successfully demonstrated the replaceability among pyrimidine dione, thienopryimidine, and pyrrolopyrimidine scaffold with similar DPP-IV inhibitory activity based on scaffold hopping strategy[11]. Later we went on hybridizing butynyl pharmacophore into our pyrrolopyrimidine scaffold and generated another potent DPP-IV inhibitor with nano malar IC_{50} value[12]. At the same time, we noticed the similar location of butynyl and cyanobenzyl in DPP-IV enzyme in a structure superposition of DPP-IV enzymes complex with Alogliptin and Linagliptin. Therefore we applied the pharmacophore hybridization directly to pyrimidine dione scaffold of Alogliptin and replaced cyanobenzyl with butynyl as our another novel hit (**61**, IC_{50}=198 nM). Herein we tried to present the generation process of compound **61** and its further optimization, as well as the pharmacological evaluations on the representative compound **77**.

2. Chemistry

The synthesis of compounds **11a-o** is outlined in **Scheme 1**. Alkylation of **8** with 1-bromo-2-butyne provided precursor **9**. Compounds **10a-o** were obtained by the N-alkylation of **9**. Compounds **11a-o** were obtained by the replacement of the chloro group with a 3-(R)-aminopiperidinyl group.



Scheme 1. Synthesis of compounds **11a-o**. Reagents: (a) 1-bromo-2-butyne, DIEA, DMF, rt; (b) RCH_2X (X = Cl, Br, I), NaH, LiBr, DMF; (c) 3-(R)-aminopiperidine, $NaHCO_3$, 120℃, $NaHCO_3$, EtOH.

3. Results and discussion

*3.1. Generation of hit compound **61**.*

Our previous work on DPP-IV inhibitors revealed that the cyanobenzyl should be freely changeable with butynyl if the 3-(R)-aminopiperidinyl group was still present[12]. In addition, we superimposed the crystal structures of DPP-4 complex with Alogliptin[6] and Linagliptin[13] in Discovery Studio 3.0 (Accelrys, San Diego, CA). We observed the butynyl and cyanobenzyl laid in the same S1 pocket of the enzyme which may indicate the replaceability of them. In the other hand, we had proved the scaffold hopping feasibility between xanthine and pyrimidine dione scaffold. As a result, compound **61** was immediately generated by replacing cyanobenzyl with butynyl directly on Alogliptin. Compound **61** showed a decreased activity with IC_{50} of 198 nM, which may partially due to the lack of interaction between butynyl and Arg 125 than that within cyanobenzyl. Yet compound **61** was still chose to go on the followed optimization for its acceptable activity and selectivity against DPP-IV.

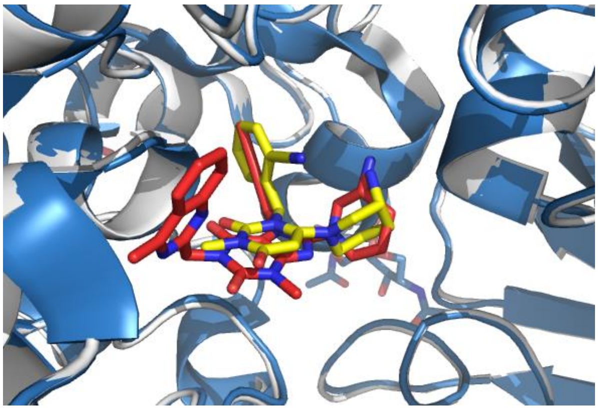


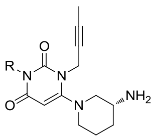
Figure 2. Structure superposition between crystal structures of DPP4 bingding Alogliptin (PDB ID. 3G0B, blue and yellow) and Linagliptin (PDB ID. 2RGU, grey and red).

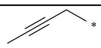
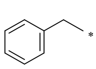
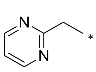
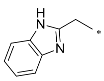
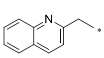
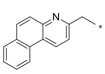
*3.2. N-3 position variations on compound **61**.*

Though with decreased activity, compound **61** has large space to improve in this issue. Compared with crystal structure of Linagliptin, variations on pyrimidine dione of **61** could easily provide another interaction site with proper substituent. Thus substitutions variation was conducted at N-3 position of compound **61** to evaluate their effect on DPP-IV inhibitory activity. Data are listed in Table 1. Inhibition on DPP-8/9 has been frequently associated with toxicity in animal studies. Thus the inhibitory activity against DPP-8/9 were concurrently evaluated. Butynyl substituted pyrimidine dione scaffold generally displayed very good selectivity *in vitro* and with low risk of toxicity[14]. Generally substitutions with proper size (**65**-**66** vs. **69**-**71**) and stereo direction (**68** vs. **69**-**71**) had a better affinity with DPP-IV enzyme. Compound **67** with pyrimidinyl has IC_{50} value of 3.4 nM. Compound **70** has the lowest IC_{50} with increase of hydrophobicity. Yet too much hydrophobicity would decrease the interaction with enzymes on the contrary (**71**).

Pharmacokinetic study of **70** showed its 63.3% oral bioavailability and 4.2 hours oral half life in male Sprague Dawley rats. The intravenous half life was 1.76 hours. The difference between oral and intravenous half life might indicate this compound bears a possibility of enterohepatic cycling, which contributes to the longer half life. Fortunately **70** has little inhibition on cytochrome P450 3A (CYP 3A, IC_{50}=97.9 μM), reducing the drug-drug interaction risk. As a result, compound **70** is selected as the lead compound for further optimization.

Table 1. DPP inhibitory profile of compound **65**-**71**.



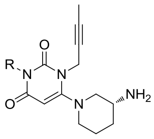
No.	R	IC_{50}		
		DPP-IV (nM)	DPP-8 (μM)	DPP-9 (μM)
6511b		2177.5	>100	>100
6611c		>10000	>100	>100
6711d		3.4	>100	>100
6911e		22.0	>100	>100
7011f		3.1	>100	>100
7111g		31.3	>100	>100

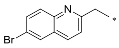
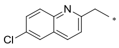
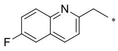
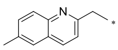
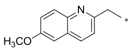
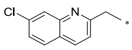
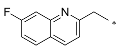
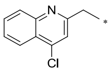
Data represent means of at least two independent experiments.

*3.3. Lead optimization on compound **70**.*

We evaluated different single substitutions on quinoline of lead compound **70**. Table 2 summarizes the inhibitory properties. From compound **72** to**76**, small and electronic donor groups provided better DPP-IV inhibitory activity at 6 position. We believe that the R part of this structure should interact with the enzyme mainly by π-π stacking. Thus the effect of electronic effect accounts for more effect than steric hindrance (**76**). Based on the data, substitutions preference showed a 7 position> 4 position> 6 position sequence. Among them, 7-chloroquinolinylmethly substituted compound **77** displayed highly potent and selective property. Although variations on 7 position were not so sufficient, electronic effect was still slightly over steric hindrance.

Table 2. Inhibitory profile of compound **72-79**.



No.	R	IC_{50}			
		DPP-IV (nM)	DPP-8 (μM)	DPP-9 (μM)	CYP 3A (μM)
7211h		10.8	>100	>100	79.36±14.78
7311i		6.6	>100	>100	91.93±30.21
7411j		2.0	>100	>100	58.28±9.47
7511k		9.1	>100	>100	46.24±8.07
7611l		1.9	>100	>100	42.19±6.19
7711m		0.36	>100	>100	46.18±6.80
7811n		0.68	>100	>100	54.65±7.25
7911o		1.23	>100	84.5	14.08±1.95
Alogliptin		1.5			>30[6]

Data represent nominalized means of at least two independent experiments.

*3.4. Pharmacological evaluations on compound **77**.*

Besides good activity and selectivity against DPP-IV, compound **77** also has low risk of drug-drug interaction and heart QT interval prolongation with IC_{50} 46.2 μM against CYP 3A and over 100 μM against hERG (Table 2). As regard to the *in vivo* evaluations, compound **77** had a fine PK profile with 73.3% bioavailability and over 5 hours half life in male rats similar to compound **70** (Table 3, Figure 3). In a 24 hours *in vivo* test, **77** displayed lower DPP-IV activity than Alogliptin (Figure 4) and the corresponding inhibition rate were calculated in Table 4. Under our experiment condition, the half life of Alogliptin in male rats was close to 2 hours. Hence, **77** contributed to longer duration of low DPP-IV activity and slower restoration.

Table 3. Selected PK parameters for compound **77** in male Sprague Dawley Rats.

Dose(mg/kg) i.v./p.o.	iv $T_{1/2}$ (h)	oral $T_{1/2}$ (h)	$poAUC_{0-t}$ (μg•h•mL^{-1})	CLp (L•h^{-1}•kg^{-1})	$V_{Z/F}$ (L•kg-1)	F%
5/25	5.65 ±1.13	5.02 ±1.39	17.58 ±2.82	1.05 ±0.15	8.65±2.43	73.3

i.v., intravenous injection; p.o., oral administration.

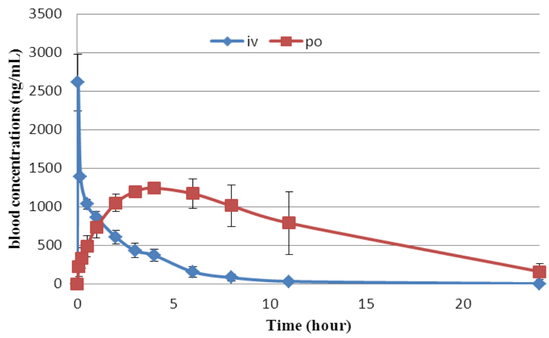


Figure 3. Concentration- time curve of compound **77**.

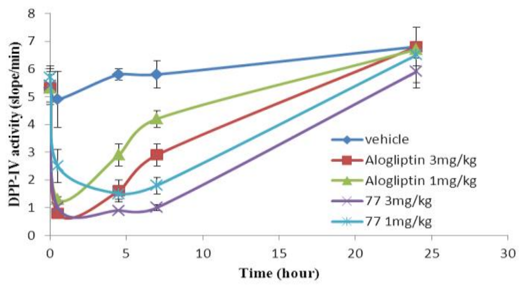


Figure 4. Effect on DPP-IV activity of compound **77** in ICR mice.

Table 4. DPP-IV inhibition of compound **77** in ICR mice.

	Dose	DPP-IV inhibition			
	mg/kg	0.5 h	4.5 h	7 h	24 h
Alogliptin	3	83.80%	71.50%	50.90%	0.80%
	1	72.60%	49.40%	27.60%	0.50%
77	3	80.40%	84.70%	82.30%	13.50%
	1	51.80%	76.00%	71.60%	11.50%

4. Conclusion

Our previous work had demonstrated the feasibility of scaffold hopping among thienopyrimidine, pyrrolopyrimidine and pyrimidine dione in developing DPP-IV inhibitors. And replacement cyanobenzyl with butynyl was proved to be changeable in our previous work and computational structure superposition. Thus butynyl substituted pyrimidine dione compound **61** (IC_{50}=198 nM) was determined as the hit compound based on Alogliptin. Variations on N-3 position of compound **61** indicated a proper hydrophobic quinolinylmethly substitution brought better affinity with DPP-IV enzyme represented by **70** (IC_{50}=3.1 nM). Further optimization on the quinoline ring demonstrated 7 position is the preferred substitution site. And electronic effect played an important role of quinoline part on DPP-IV inhibitory activity which might due to π-π stacking interaction rather than steric hindrance. Eventually, in this work, the most potent and oral bioavailable DPP-IV inhibitor **77** (IC_{50}=0.36 nM, F=77.3%, $T_{1/2}$= 5 h) was proved to bear a better *in vivo* DPP-IV activity decrease than Alogliptin for a longer duration.

5. Experimental section

5.1. Chemistry

All commercially available compounds and solvents were of reagent grade and used without further treatment unless otherwise noted. Reactions were monitored by TLC using Qing Dao Hai Yang GF254 silica gel plates (5 x 10 cm); zones were detected visually under ultraviolet irradiation (254 nm) and by spraying with an ethanol solution of 2,4-DNP or ninhydrin, or by fuming with iodine steam. Silica gel column chromatography was performed on silica gel (200-300 mesh) from Qing Dao Hai Yang. NMR spectra were recorded on a Bruker NMR AVANCE 400 (400 MHz) or a Bruker NMR AVANCE 500 (500 MHz). Chemical shifts (δ) were recorded in ppm and coupling constants (J) in hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s

(singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or br (broad). MS data were measured on an Agilent MSD-1200 ESI-MS system.

5.1.1. 1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1H,3H)-dione (9).

1-bromo-2-butyne (9.4 mL, 0.11mol) was added to a mixture of 6-chloropyrimidine-2,4(1H,3H)-dione (14.6g, 0.1mol) , ethyldiisopropylamine (15 mL, 0.15mol) and 250 mL of N,N-dimethylformamide. The reaction mixture is stirred overnight at ambient temperature. For working up the reaction mixture is diluted with approx. 300 mL of water. The light precipitate formed is suction filtered and washed with water. The filter cake is washed with diethyl ether and dried to give 1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1H,3H)-dione **(9)** as a yellow powder (17 g, yield 85%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.91 (s, 1H), 4.75 (d, *J* = 2.0 Hz, 2H), 1.82 (t, *J* = 2.0 Hz, 3H). ESI-MS calculated for (C8H8ClN2O2) $[M+H]^+$, 199.03, found 199.0.

General procedure for the synthesis of compounds 10a-o.

5.1.2. 1-(but-2-yn-1-yl)-6-chloro-3-methylpyrimidine-2,4(1H,3H)-dione (10a).

A mixture of **9** (500 mg, 2.5 mmol) and 5mL of DMF was added NaH (60% in oil, 2eq) at 0 ℃ and LiBr(3eq) after stirred for 5min. The reaction mixture was subsequently cooled to 0 ℃ after stirred for 20min and added CH_3I (900 mg, 6.2 mmol) at 0 ℃. The reaction mixture was stirred overnight at room temperature. The mixture was poured into 50 ml of water and extracted with ethyl acetate. The combined organic layers was washed with water and brine and dried over magnesium sulfate. After filtration the solvent is removed and the crude residue purified by silica gel column chromatography to give 525 mg (98%) of the title compound. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.94 (s, 1H), 4.77 (d, *J* = 2.4 Hz , 2H), 3.33 (s, 3H), 1.81-1.80 (t, *J* = 2.4 Hz, 3H). ESI-MS calculated for (C9H10ClN2O2) $[M+H]^+$, 213.04, found 213.0.

5.1.3. (R)-6-(3-aminopiperidin-1-yl)-1,3-di(but-2-yn-1-yl)pyrimidine-2,4(1H,3H)-dione (10b).

The title compound was prepared from 1-bromobut-2-yne in 90.0% yield according to the procedure for example **10a**. ^{1}H-NMR (400MHz, $CDCl_3$) δ 5.96 (s, 1H), 4.80-4.78 (q, *J* = 2.4 Hz, 2H), 4.64-4.62 (q, *J* = 2.4 Hz, 2H), 1.82-1.81 (t, *J* = 2.4 Hz, 3H), 1.78-1.77 (t, *J* = 2.4 Hz, 3H). ESI-MS calculated for (C12H12ClN2O2) $[M+H]^+$, 251.06, found 251.0.

5.1.4. 3-benzyl-1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1H,3H)-dione (10c).

The title compound was prepared from (bromomethyl)benzene in 76.0% yield according to the procedure for example **10a**. ^{1}H-NMR (400MHz, $CDCl_3$) δ 7.40-7.38 (m, 2H), 7.26-7.14 (m, 3H), 5.86 (s, 1H), 4.99 (s, 2H), 4.65-4.64 (d, *J* = 2.4 Hz, 2H), 1.73-1.72 (t, *J* = 2.4 Hz, 3H). ESI-MS calculated for (C15H14ClN2O2) $[M+H]^+$, 289.07, found 289.1.

5.1.4. 1-(but-2-yn-1-yl)-6-chloro-3-(pyrimidin-2-ylmethyl)pyrimidine-2,4(1H,3H)-dione (10d).

The title compound was prepared from 2-(chloromethyl)pyrimidine in 71.0% yield according to the procedure for example **8a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.56 (d, *J* = 4.8 Hz, 2H), 7.09 (t, *J* = 4.8 Hz, 1H), 5.94 (s, 1H), 5.28 (s, 2H), 4.72-4.71 (m, 2H), 1.73 (t, *J* = 2.4 Hz, 3H). ESI-MS calculated for (C13H12ClN4O2) $[M+H]^+$, 291.06, found 291.0.

5.1.5. 3-((1H-benzo[d]imidazol-2-yl)methyl)-1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1H,3H)-dione (10e).

The title compound was prepared from 2-(chloromethyl)-1H-benzo[d]imidazole in 27.6% yield according to the procedure for example **10a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.59-7.56 (m, 2H), 7.24-7.21 (m, 2H), 5.99 (s, 1H), 5.40 (s, 2H), 4.75 (d, *J* = 2.4 Hz, 2H), 1.79 (t, *J* = 2.4 Hz, 3H). ESI-MS calculated for (C16H14ClN4O2) $[M+H]^+$,

329.08, found 329.1.

5.1.6. 1-(but-2-yn-1-yl)-6-chloro-3-(quinolin-2-ylmethyl)pyrimidine-2,4(1H,3H)-dione (10f).

The title compound was prepared from 2-(chloromethyl)quinoline in 23.9% yield according to the procedure for example **8a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 8.4 Hz, 1H), 8.00 (d, *J* = 8.4 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.67-7.63 (m, 1H), 7.50-7.46 (m, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 6.05 (s, 1H), 5.43 (s, 2H), 4.82-4.80 (m, 2H), 1.82 (t, *J* = 2.4 Hz, 3H). ESI-MS calculated for (C18H15ClN3O2) $[M+H]^+$, 340.08, found 340.1.

5.1.7.3-(benzo[f]quinolin-3-ylmethyl)-1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1H,3H)-dione (10g).

The title compound was prepared from 3-(chloromethyl)benzo[f]quinoline in 33.1% yield according to the procedure for example **10a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.89 (d, *J* = 8.8 Hz, 1H), 8.58 (d, *J* = 8.0 Hz, 1H), 7.95-7.90 (m, 3H), 7.69-7.60 (m, 2H), 7.49 (d, *J* = 8.4 Hz, 1H), 6.06 (s, 1H), 5.48 (s, 2H), 4.81 (d, *J* = 2.4 Hz, 2H), 1.83 (t, *J* = 2.4 Hz, 3H). ESI-MS calculated for (C22H17ClN3O2) $[M+H]^+$, 390.10, found 390.1.

5.1.8. 3-((6-bromoquinolin-2-yl)methyl)-1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1H,3H)-dione (10h).

The title compound was prepared from 6-bromo-2-(chloromethyl)quinoline in 32.4% yield according to the procedure for example **8a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.8 Hz, 1H), 7.93 (d, *J* = 2.0 Hz, 1H), 7.86 (d, *J* = 8.8 Hz, 1H), 7.72-7.69 (m, 1H), 7.34 (d, *J* = 8.4Hz, 1H), 6.05 (s, 1H), 5.40 (s, 2H), 4.81-4.80 (m, 2H), 1.82 (t, *J* = 2.4 Hz, 3H). ESI-MS calculated for (C18H14BrClN3O2) $[M+H]^+$, 418.00,419.99, found 419.0, 421.00.

5.1.9. 1-(but-2-yn-1-yl)-6-chloro-3-((6-chloroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (10i).

The title compound was prepared from 6-chloro-2-(chloromethyl)quinoline in 53.7% yield according to the procedure for example **10a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.4 Hz, 1H), 7.92 (d, *J* = 8.8 Hz, 1H), 7.74 (d, *J* = 2.4 Hz, 1H), 7.58-7.56 (m, 1H), 7.34 (d, *J* = 8.4 Hz, 1H), 6.04 (s, 1H), 5.40 (s, 2H), 4.81-4.79 (m, 2H), 1.82 (t, *J* = 2.4 Hz, 3H). ESI-MS calculated for (C18H14Cl2N3O2) $[M+H]^+$, 374.05, 376.04, found 374.0, 376.0.

5.1.10. 1-(but-2-yn-1-(but-2-yn-1-yl)-6-chloro-3-((6-fluoroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (10j).

The title compound was prepared from 2-(chloromethyl)-6-fluoroquinoline in 58.9% yield according to the procedure for example **10a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.4 Hz, 1H), 7.99 (dd, *J* = 5.2 Hz, 1H), 7.44-7.34 (m, 3H), 6.05 (s, 1H), 5.41 (s, 2H), 4.82-4.80 (m, 2H), 1.82 (t, *J* = 2.0 Hz, 3H). ESI-MS calculated for (C18H14ClFN3O2) $[M+H]^+$, 358.08, found 358.1.

5.1.11. 1-(but-2-yn-1-yl)-6-chloro-3-((6-methylquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (10k).

The title compound was prepared from 2-(chloromethyl)-6-methylquinoline in 51.9% yield according to the procedure for example **10a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.4 Hz, 1H), 7.95 (d, *J* = 8.8 Hz, 1H), 7.87(d, *J* = 1.6 Hz, 1H), 7.80-7.77 (m, 1H), 7.26 (d, *J* = 8.4 Hz, 1H), 5.99 (s, 1H), 5.25 (s, 2H), 4.77-4.75 (m, 2H), 2.72 (s, 3H), 1.80 (t, *J* = 2.4 Hz, 3H). ESI-MS calculated for (C19H17ClN3O2) $[M+H]^+$, 354.10, found 354.1.

5.1.12. 1-(but-2-yn-1-yl)-6-chloro-3-((6-methoxyquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (10l).

The title compound was prepared from 2-(chloromethyl)-6-methoxyquinoline in 55.1% yield according to the procedure for example **10a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 9.2 Hz, 1H), 7.31-7.27 (m, 2H), 7.03 (d, *J* = 2.8 Hz, 1H), 6.04 (s, 1H), 5.39 (s, 2H), 4.81-4.79 (m, 2H), 3.90 (s, 3H), 1.82 (t, *J* = 2.4 Hz, 3H). ESI-MS calculated for (C19H17ClN3O3) $[M+H]^+$, 370.10, found 370.1.

5.1.13. 1-(but-2-yn-1-yl)-6-chloro-3-((7-chloroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (10m).

The title compound was prepared from 7-chloro-2-(chloromethyl)quinoline in 58.8% yield according to the procedure for example **10a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 8.4 Hz, 1H), 8.00 (s, 1H), 7.69 (d, *J* = 8.8 Hz, 1H), 7.43-7.41 (m, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 6.04 (s, 1H), 5.40 (s, 2H), 4.81-4.80 (m, 2H), 1.83 (t, *J* = 2.4 Hz, 3H). ESI-MS calculated for (C18H14Cl2N3O2) $[M+H]^+$, 374.05, 376.04, found 374.0, 376.0.

5.1.14. 1-(but-2-yn-1-yl)-6-chloro-3-((7-fluoroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (10n).

The title compound was prepared from 2-(chloromethyl)-7-fluoroquinoline in 42.7% yield according to the procedure for example **10a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 8.8 Hz, 1H), 7.77-7.73 (m, 1H), 7.63-7.60 (m, 1H), 7.30-7.25 (m, 2H), 6.05 (s, 1H), 5.41 (s, 2H), 4.82-4.81 (m, 2H), 1.83 (t, *J* = 2.4 Hz, 3H). ESI-MS calculated for (C18H14ClFN3O2) $[M+H]^+$, 358.08, found 358.1.

5.1.15. 1-(but-2-yn-1-yl)-6-chloro-3-((4-chloroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (10o).

The title compound was prepared from 4-chloro-2-(chloromethyl)quinoline in 58.0% yield according to the procedure for example **10a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.17 (d, *J* = 8.0 Hz, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.72-7.68 (m, 1H), 7.60-7.56 (m, 1H), 7.41 (s, 1H), 6.05 (s, 1H), 5.39 (s, 2H), 4.81-4.80 (m, 2H), 1.82 (t, *J* = 2.4 Hz, 3H). ESI-MS calculated for (C18H14Cl2N3O2) $[M+H]^+$, 374.05, 376.04, found 374.0, 376.0.

General Procedure for the Synthesis of Compounds 11a-p.

5.1.16. (R)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-methylpyrimidine-2,4(1H,3H)-dione (11a).

A mixture of **10a** (200 mg, 0.94 mmol), (*R*)-3-aminopiperdine dihydrochloride (244 mg, 1.41 mmol) and sodium bicarbonate (395 mg, 4.7 mmol) were stirried with 100 mg activated MS (4A) in dry ethanol (10 mL) at 100℃ for 2h. The reaction concentrated in vacuo, and purified by flash chromatography to afford 169 mg (65.2%) of the title compound. ^{1}H-NMR (400 MHz, MeOD) δ 5.32 (s, 1H), 4.67-4.57 (m, 2H), 3.38-3.36 (m, 2H), 3.32 (s, 3H), 3.05-2.98 (m, 1H), 2.84-2.79 (m, 1H), 2.61-2.56 (m, 1H), 2.08-2.04 (m, 1H), 1.96-1.91 (m, 1H), 1.87 (t, *J* = 2.4 Hz, 3H), 1.83-1.74 (m, 1H), 1.44-1.35 (m, 1H); ^{13}C-NMR (125 MHz, MeOD) δ 165.41, 161.55, 153.75, 89.32, 80.97, 74.80, 59.41, 52.82, 48.49, 36.62, 33.47, 28.12, 24.33, 3.07. ESI-MS calculated for (C14H21N4O2) $[M+H]^+$, 277.17, found 277.2.

5.1.17. (R)-6-(3-aminopiperidin-1-yl)-1,3-di(but-2-yn-1-yl)pyrimidine-2,4(1H,3H)-dione (11b).

The title compound was prepared from 1,3-di(but-2-yn-1-yl)-6- chloropyrimidine-2,4(1H,3H)-dione (**10b**) in 68.2% yield according to the procedure for example **11a**. ^{1}H-NMR (400 MHz, MeOD) δ 5.32 (s, 1H), 4.47-4.58 (m, 4H), 3.41-3.40 (m, 1H), 3.37-3.36 (m, 1H), 3.05-2.98 (m, 1H), 2.86-2.81 (m, 1H), 2.63-2.58 (m, 1H), 2.08-2.04 (m, 1H), 1.97-1.93 (m, 1H), 1.87 (t, *J* = 2.0 Hz, 3H), 1.81 (t, *J* = 2.0 Hz, 3H), 1.78-1.74 (m, 1H), 1.45-1.35 (m, 1H); ^{13}C-NMR (125 MHz, MeOD) δ 164.15, 161.83, 152.98, 89.32, 81.17, 78.93, 74.73, 74.55, 59.37, 52.79, 48.47, 36.77, 33.45, 31.57, 24.31, 3.11, 3.05. ESI-MS calculated for (C17H23N4O2) $[M+H]^+$, 315.18, found 315.2.

5.1.18. (R)-6-(3-aminopiperidin-1-yl)-3-benzyl-1-(but-2-yn-1-yl)pyrimidine-2,4(1H,3H)-dione (11c).

The title compound was prepared from 3-benzyl-1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1H,3H)-dione (**10c**) in 67.0% yield according to the procedure for example **11a**. ^{1}H-NMR (400 MHz, MeOD) δ 7.35-7.31 (m, 2H), 7.29-7.22 (m, 3H), 5.30 (s, 1H), 5.07 (s, 2H), 4.56 (t, *J* = 18.4 Hz, 2H), 3.37-3.33 (m, 2H), 2.96-2.93 (m, 1H),

2.81-2.73 (m, 1H), 2.57-2.52 (m, 1H), 2.02-1.99 (m, 1H), 1.89-1.86 (m, 1H), 1.81 (s, 3H), 1.76-1.71 (m, 1H), 1.38-1.31 (m, 1H); ^{13}C-NMR (125 MHz, MeOD) δ 165.10, 161.72, 153.66, 138.42, 129.35(2C), 129.15(2C), 128.41, 89.37, 81.10, 74.71, 59.36, 52.75, 48.45, 45.22, 36.83, 33.45, 24.31, 3.06. ESI-MS calculated for (C20H25N4O2) $[M+H]^+$, 353.20, found 353.2.

5.1.19. (R)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-(pyrimidin-2-ylmethyl)pyrimidine-2,4(1H,3H)-dione (11d).

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-(pyrimidin-2-ylmethyl)pyrimidine-2,4(1H,3H)-dione (**10d**) in 69.4% yield according to the procedure for example **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 4.8 Hz, 2H), 7.09 (t, J = 4.8 Hz, 1H), 5.32(s, 2H), 5.25 (s, 1H), 4.51 (d, J = 2.0 Hz, 2H), 3.60-3.33 (m, 1H), 3.25-3.22 (m, 1H), 3.01-2.97 (m, 1H), 2.73-2.71 (m, 1H), 2.55-2.45 (m, 1H), 1.97-1.93 (m, 1H), 1.86-1.82 (m, 1H), 1.76 (s, 3H), 1.68-1.60 (m, 1H), 1.30-1.25 (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 165.36, 163.07, 159.52, 157.17, 152.26, 119.30, 89.04, 80.25, 73.64, 56.72, 51.65, 47.50, 46.10, 35.78, 31.07, 22.94, 3.63; ESI-MS calculated for (C18H23N6O2) $[M+H]^+$, 355.19, found 355.2.

5.1.20.(R)-3-((1H-benzo[d]imidazol-2-yl)methyl)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)pyrimidine-2,4(1H,3H)-dione (11e).

The title compound was prepared from 3-((1H-benzo[d]imidazol-2-yl)methyl)-1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1H,3H)-dione (**10e**) in 35.1% yield according to the procedure for example **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.57-7.41 (m, 2H), 7.19-1.12 (m, 2H), 5.31 (s, 2H), 5.18 (s, 1H), 4.40 (s, 2H), 3.22-3.19 (m, 3H), 3.01-2.95 (m, 1H), 2.66-2.59 (m, 1H), 2.46-2.40 (m, 1H), 1.98-1.88 (m, 1H), 1.75 (s, 3H), 1.64-1.55 (m, 1H), 1.32-1.26 (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$+MeOH) δ 162.87, 159.64, 151.96, 149.77, 137.81, 122.36, 114.73, 88.57, 80.37, 73.26, 56.36, 51.30, 49.82, 47.13, 35.71, 30.82, 22.63, 3.25; ESI-MS calculated for (C21H25N6O2) $[M+H]^+$, 393.20, found 393.20.

5.1.21.(R)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-(quinolin-2-ylmethyl)pyrimidine-2,4(1H,3H)-dione (11f).

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-(quinolin-2-ylmethyl)pyrimidine-2,4(1H,3H)-dione (**10f**) in 89.7% yield according to the procedure for example **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 8.8 Hz, 1H), 8.00 (d, J = 8.8 Hz, 1H), 7.73 (d, J = 8.0 Hz, 1H), 7.62 (t, J = 7.2 Hz, 1H), 7.44 (t, J = 7.2 Hz, 1H), 7.29 (t, J = 8.4 Hz, 1H), 5.40 (s, 2H), 5.28 (s, 1H), 4.53 (d, J = 2.4 Hz, 2H), 3.35-3.32 (m, 1H), 3.24-3.21 (m, 1H), 3.02-2.98 (m, 1H), 2.73-2.71 (m, 1H), 2.55-2.46 (m, 1H), 1.97-1.93 (m, 1H), 1.79-1.76 (m, 1H), 1.72 (s, 3H), 1.71-1.61 (m, 1H), 1.31-1.23 (m, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ 163.04, 159.51, 156.49, 152.39, 147.65, 136.56, 129.26, 129.21, 127.37, 127.25, 126.02, 119.09, 89.05, 80.15, 73.68, 58.42, 51.48, 47.47, 46.47, 35.65, 32.44, 23.09, 3.59; ESI-MS calculated for (C23H26N5O2) $[M+H]^+$, 404.21, found 404.2.

5.1.22.(R)-6-(3-aminopiperidin-1-yl)-3-(benzo[f]quinolin-3-ylmethyl)-1-(but-2-yn-1-yl)pyrimidine-2,4(1H,3H)-dione (11g).

The title compound was prepared from 3-(benzo[f]quinolin-3-ylmethyl)-1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1H,3H)-dione (**10g**) in 85.8% yield according to the procedure for example **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.77 (d, J = 8.8 Hz, 1H), 8.46 (d, J = 8.0 Hz, 1H), 7.88-7.80 (m, 3H), 7.58-7.50 (m, 2H), 7.41 (d, J = 8.4 Hz, 1H), 5.44 (s, 2H), 5.24 (s, 1H), 4.48 (d, J =

2.0 Hz, 2H), 3.26-3.23 (m, 1H), 3.17-3.14 (m, 1H), 2.92-2.87 (m, 1H), 2.64-2.59 (m, 1H), 2.43-2.39 (m, 1H), 1.88-1.84 (m, 1H), 1.77-1.76 (m, 1H), 1.73 (s, 3H), 1.62-1.53 (m, 1H), 1.22-1.16 (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 163.08, 159.48, 156.06, 152.35, 147.63, 131.46, 131.25, 130.58, 129.51, 128.53, 128.19, 126.90, 124.15, 122.47, 119.13, 89.05, 80.19, 73.66, 57.83, 51.51, 47.46, 46.29, 35.70, 31.98, 23.02, 3.61; ESI-MS calculated for (C27H28N5O2) $[M+H]^+$, 454.22, found 454.2.

5.1.23.(R)-6-(3-aminopiperidin-1-yl)-3-((6-bromoquinolin-2-yl)methyl)-1-(but-2-yn-1-yl)pyrimidine-2,4(1H,3H)-dione (11h).

The title compound was prepared from 3-((6-bromoquinolin-2-yl)methyl)-1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1H,3H)-dione (**10h**) in 86.3% yield according to the procedure for example **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.8 Hz, 1H), 7.88-7.84 (m, 2H), 7.68-7.65 (m, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 5.37 (s, 2H), 5.28 (s, 1H), 4.54 (d, *J* = 2.4 Hz, 2H), 3.36-3.34 (m, 1H), 3.25-3.22 (m, 1H), 3.04-2.98 (m, 1H), 2.75-2.70 (m, 1H), 2.54-2.51 (m, 1H), 1.98-1.94 (m, 1H), 1.88-1.83 (m, 1H), 1.77 (t, *J* = 2.4 Hz, 3H), 1.74-1.64 (m, 1H), 1.33-1.27 (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 162.99, 159.51, 156.92, 152.23, 146.03, 135.50, 132.58, 130.79, 129.31, 128.22, 119.99, 119.70, 88.83, 80.14, 73.55, 57.58, 51.43, 47.36, 46.25, 35.68, 31.77, 22.91, 3.52; ESI-MS calculated for (C23H25BrN5O2) $[M+H]^+$, 482.12, 484.12, found 482.1, 484.1.

5.1.24.(R)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((6-chloroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (11i).

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-((6-chloroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (1**0i**) in 82.9% yield according to the procedure for example **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.8 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.70 (d, *J* = 2.4 Hz, 1H), 7.55-7.52 (m, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 5.37 (s, 2H), 5.28 (s, 1H), 4.53 (d, *J* = 2.4 Hz, 2H), 3.35-3.32 (m, 1H), 3.24-3.21 (m, 1H), 3.03-2.97 (m, 1H), 2.74-2.69 (m, 1H), 2.53-2.50 (m, 1H), 1.97-1.93 (m, 1H), 1.87-1.82 (m, 1H), 1.76 (t, *J* = 2.4 Hz, 3H), 1.71-1.64 (m, 1H), 1.31-1.26 (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 162.98, 159.44, 156.71, 152.15, 145.76, 135.57, 131.50, 130.59, 130.00, 127.64, 125.91, 119.96, 88.76, 80.11, 73.49, 57.06, 51.40, 47.29, 46.17, 35.67, 31.34, 22.82, 3.47; ESI-MS calculated for (C23H25ClN5O2) $[M+H]^+$, 438.17, found 438.2.

5.1.25.(R)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((6-fluoroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (11j).

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-((6-fluoroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**10j**) in 88.1% yield according to the procedure for example **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.99-7.96 (m, 2H), 7.40-7.29 (m, 3H), 5.37 (s, 2H), 5.28 (s, 1H), 4.53 (d, *J* = 2.4 Hz, 2H), 3.35-3.32 (m, 1H), 3.24-3.21 (m, 1H), 3.03-2.98 (m, 1H), 2.73-2.68 (m, 1H), 2.52-2.50 (m, 1H), 1.97-1.93 (m, 1H), 1.87-1.82 (m, 1H), 1.77 (t, *J* = 2.0 Hz, 3H), 1.71-1.61 (m, 1H), 1.31-1.26 (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$+MeOH) δ 163.12, 159.99, 159.52, 155.65, 152.15, 144.30, 136.11, 131.02, 127.67, 119.74, 119.43, 110.30, 88.76, 80.22, 73.26, 57.17, 51.42, 47.18, 46.04, 35.57, 31.41, 22.76, 3.27; ESI-MS calculated for (C23H25FN5O2) $[M+H]^+$, 422.20, found 422.2.

5.1.26.(R)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((6-methylquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (11k).

The title compound was prepared from

1-(but-2-yn-1-yl)-6-chloro-3-((6-methylquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**10k**) in 82.1% yield according to the procedure for example **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 8.4 Hz, 1H), 7.92-7.87 (m, 2H), 7.82-7.79 (m, 1H), 7.21 (d, J = 8.4 Hz, 1H), 5.23-5.22 (m, 3H), 4.49 (d, J = 1.2 Hz, 2H), 3.31-3.28 (m, 1H), 3.20-3.17 (m, 1H), 3.01-2.94 (m, 1H), 2.68 (s, 3H), 2.48-2.46 (m, 1H), 1.96-1.92 (m, 1H), 1.85-1.80 (m, 1H), 1.78 (t, J = 2.4 Hz, 3H), 1.71-1.59 (m, 2H), 1.29-1.27 (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 162.86, 159.23, 158.70, 152.02, 146.98, 136.11, 134.27, 130.53, 128.27, 127.65, 125.98, 121.87, 88.83, 80.14, 73.48, 57.97, 51.29, 47.29, 43.98, 35.52, 32.08, 25.04, 22.91, 3.47; ESI-MS calculated for (C24H28N5O2) $[M+H]^+$, 418.22, found 418.2.

5.1.29.(R)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((6-methoxyquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (11l).

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-((6-methoxyquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**10l**) in 88.4% yield according to the procedure for example **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 9.2 Hz, 1H), 7.28-7.24 (m, 2H), 7.00 (d, J = 2.4 Hz, 1H), 5.37 (s, 2H), 5.28 (s, 1H), 4.53 (d, J = 1.6 Hz, 2H), 3.86 (s, 3H), 3.34-3.32 (m, 1H), 3.24-3.21 (m, 1H), 3.03-2.99 (m, 1H), 2.73-2.68 (m, 1H), 2.53-2.50 (m, 1H), 1.98-1.94 (m, 1H), 1.86-1.83 (m, 1H), 1.78 (s, 3H), 1.69-1.65 (m, 1H), 1.29-1.27 (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 163.06, 159.39, 157.35, 153.88, 152.29, 143.63, 135.34, 130.48, 128.13, 121.84, 119.42, 104.93, 88.97, 80.11, 73.63, 57.47, 55.36, 51.46, 47.39, 46.27, 35.65, 31.68, 22.94, 3.55; ESI-MS calculated for (C24H28N5O3) $[M+H]^+$, 434.22, found 434.2.

5.1.28.(R)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((7-chloroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (11m).

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-((7-chloroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**10m**) in 66.4% yield according to the procedure for example **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 8.4 Hz, 1H), 7.99 (s, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.41-7.39 (m, 1H), 7.30 (d, J = 8.4 Hz, 1H), 5.39 (s, 2H), 5.30 (s, 1H), 4.56 (d, J = 2.0 Hz, 2H), 3.38-3.36 (m, 1H), 3.27-3.24 (m, 1H), 3.06-3.02 (m, 1H), 2.77-2.72 (m, 1H), 2.56-2.54 (m, 1H), 1.99-1.96 (m, 1H), 1.90-1.86 (m, 1H), 1.79 (s, 3H), 1.75-1.70 (m, 1H), 1.34-1.29 (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 162.97, 159.53, 157.48, 152.35, 147.94, 136.27, 134.96, 128.57, 128.25, 126.97, 125.56, 119.40, 88.96, 80.13, 73.62, 58.45, 51.47, 47.44, 46.16, 35.61, 32.47, 23.07, 3.55; ESI-MS calculated for (C23H25ClN5O2) $[M+H]^+$, 438.17, found 438.2.

5.1.29.(R)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((7-fluoroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (11n).

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-((7-fluoroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**10n**) in 92.5% yield according to the procedure for example **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 8.8 Hz, 1H), 7.75-7.71 (m, 1H), 7.64-7.61 (m, 1H), 7.28-7.22 (m, 2H), 5.40 (s, 2H), 5.31 (s, 1H), 4.56 (d, J = 1.6 Hz, 2H), 3.38-3.36 (m, 1H), 3.27-3.24 (m, 1H), 3.06-3.02 (m, 1H), 2.77-2.72 (m, 1H), 2.56-2.54 (m, 1H), 2.00-1.97 (m, 1H), 1.90-1.86 (m, 1H), 1.80 (s, 3H), 1.75-1.72 (m, 1H), 1.34-1.29 (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 162.96, 162.85, 159.50, 157.52, 152.30, 148.52, 136.36, 129.31, 124.16, 118.40, 116.35, 112.80, 88.91, 80.10, 73.59, 58.11, 51.44, 47.40, 46.22, 35.61, 32.19, 23.00, 3.51; ESI-MS calculated for (C23H25FN5O2) $[M+H]^+$, 422.20, found 422.2.

5.1.30.(R)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((4-chloroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)

-dione (11o).

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-((4-chloroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**10o**) in 71.9% yield according to the procedure for example **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 8.4 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.68(t, J = 7.6 Hz, 1H), 7.55 (t, J = 7.6 Hz, 1H), 7.38 (s, 1H), 5.37 (s, 2H), 5.30 (s, 1H), 4.55 (s, 2H), 3.38-3.35 (m, 1H), 3.26-3.24 (m, 1H), 3.08-2.97 (m, 1H), 2.82-2.67 (m, 1H), 2.60-2.47 (m, 1H), 1.98-1.96 (m, 1H), 1.88-1.87 (m, 1H), 1.79 (s, 3H), 1.75-1.65 (m, 1H), 1.34-1.26 (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 162.87, 159.59, 156.62, 152.35, 148.49, 142.98, 130.19, 129.63, 127.05, 125.47, 123.80, 119.16, 89.01, 80.28, 73.58, 58.68, 51.49, 47.49, 46.22, 35.72, 32.66, 23.12, 14.06, 3.60; ESI-MS calculated for (C23H25ClN5O2) $[M+H]^+$, 438.17, found 438.17.

5.2. In vitro inhibition of DPP-IV, DPP-8 and DPP-9

Solutions of test compounds at varying concentrations（≤10 mM final concentration) were prepared in dimethyl sulfoxide (DMSO) and diluted into assay buffer containing 20 mM Tris (pH 7.4), 20 mM KCl, and 0.1mg/mL BSA. Human DPP-IV (0.1 nM final concentration) was added to the dilutions and pre-incubated for 10 minutes at ambient temperature before the reaction was initiated by the addition of Gly-Pro-AMC (H-glycyl-prolyl-7-amino-4-methylcoumarin, Sigma-Aldrich, 10μM final concentration). The total volume of the reaction mixture was 100 μL. The kinetics of the reaction was monitored (excitation at 400 nm, emission at 505 nm) for 5-10 minutes, or an endpoint was measured after 10 minutes. Inhibition constants (IC_{50}) were calculated from enzyme progress curves using standard mathematical models.

5.3 In vivo pharmacokinetic study

Adult male SD rats (n= 4/group) were administered the test compounds dissolved in distilled water at a single dose of 20 mg/kg or 25 mg/kg for oral administration and 5 mg/mL by injection. Blood samples of 100-200 μL were collected from the orbit at 11 time points within 24 hours. The blood concentration of test compounds was determined by LC-MS/MS. The PK parameters were obtained from the pharmacokinetic software DAS. 2.0.

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